ABSTRACTS

American Society for Microbiology 101 General Meeting

May 20-24, 2001

Orange County Convention Center

Orlando, Florida

AMERICAN SOCIETY
FOR MICROBIOLOGY
WASHINGTON, DC

revealed that 13 of the NT bovine mastitis strains from Argentina did not carry either the *cap5* or *cap8* locus. However, Southern blots and PCR analysis showed that all 13 strains did carry the housekeeping genes flanking the *cap5/8)* gene cluster. PCR amplification and sequencing of the DNA between the housekeeping genes in these NT strains is ongoing. Our results indicate that some NT *S. aureus* isolates fail to produce CP5 or CP8 because they lack the *cap5/8* gene clusters. Other strains carry an intact capsule gene cluster, and *cap*-specific mRNA could be detected in post-exponential cultures of these strains. Whether lack of CP expression by NT *S. aureus* with an intact capsule locus is due to the presence of point mutations within the *cap* cluster or regulatory mechanisms remains to be elucidated.

D-38. Binding of Fibrinogen to Serum Opacity Factor

H. S. Courtney1,2, J. B. Dale3,2, D. L. Hasty3,2
1Veterans Affairs Medical Center, Memphis, TN; 2University of Tennessee, Memphis; 3Veterans Affairs Medical Center, Memphis, TN

Serum opacity factor (SOF) is a large, extracellular, and cell-bound protein of group A streptococci that opacifies mammalian serum. SOF has two functionally distinct domains, one that opacifies serum and one that binds fibronectin. No other function has been described for SOF. In this report we describe a new function of SOF. Biotinylated fibrinogen bound to purified recombinant SOF in an enzyme-linked immunoassay. Utilizing purified, truncated recombinant SOF proteins, the fibrinogen-binding domain was localized to a region in the C-terminus of SOF. To determine which subunit of fibrinogen reacts with SOF, fibrinogen was subjected to SDS-PAGE under reducing conditions, transferred to nitrocellulose and reacted with SOF. Binding of SOF was visualized with antiserum to SOF and DAB substrate. SOF was found to bind primarily to the Beta subunit of fibrinogen. To determine if SOF on the surface of group A streptococci plays a role in the binding of fibrinogen, the reaction of fibrinogen with a SOF-positive strain of group A streptococci was compared to that with an isogenic, SOF-negative mutant. The results suggest that surface bound SOF does bind fibrinogen. The significance of this interaction is not known, but binding of plasma proteins to bacteria contributes to the virulence of a variety of bacteria including group A streptococci. Previous work demonstrated that SOF is a virulence factor of group A streptococci, whether the interaction of SOF with fibrinogen contributes to this remains to be determined.

D-39. Characterization of Crossreactive Human T Cell Clones Produced Against Streptococcal Recombinant M6 (rM6) Protein

N. M. J. Mertens1, Y. Li1, V. A. Fischetti2, M. W. Cunningham1
1University of Oklahoma Health Sciences Center, Oklahoma City, OK; 2Rockfeller University, New York, NY

Rheumatic fever is an autoimmune sequela of group A streptococcal infection. Autoreactive antibodies and T cells are potentially involved in development of the disease. Numerous studies of crossreactive antibodies have been reported, but less is known about the T cells which are potentially involved in rheumatic heart disease. Therefore, crossreactive human T cell clones responsive to rM6 protein and human cardiac myosin were derived from a patient with previous rheumatic heart disease. Approximately 20 T cell clones were investigated for fine epitope specificity utilizing overlapping peptides of M protein and human cardiac light meromyosin (LMM). Dominant M protein peptides recognized by the T cell clones were located in the B repeat region. Three LMM peptides were recognized by the T cell clones, which also recognized human cardiac myosin, tropomyosin, and laminin. Crossreactivity increased with increased stimulation index. Most of the T cell clones were CD4+, while four were CD8+ and two were γδ positive. The crossreactive response was MHC restricted to DR or DQ. Clones were sorted using V-beta T cell receptor specific antibody to achieve 99-100 percent homogeneous population. It was interesting that the cross-reactive T cell clones had very similar epitope recognition patterns while they exhibited very broad T cell receptor V-beta gene usage (Vb13.1, Vb5.3, Vb5.1, Vb2.3, Vb6.7, Vb17, Vb18, Vb23). V-beta T cell receptor genes were analyzed for nucleotide sequence and were found to contain heterogeneous CDR3 sequences. In summary, the rM6 responsive human T cell clones were crossreactive with peptides of human cardiac myosin and streptococcal M protein, and recognition of multiple antigenic sequences was not limited to a specific group of T cell receptor sequences.

D-40. High Level Aminoglycoside-Resistant (HLAR) Isolates of Oral Streptococci and Aerococcus from Blood Specimens

A. Kanayama1, K. Matsuzaki2, I. Kobayashi1, A. Kaneko2, J. Sasaki2
1Chemotherapy Division Mitsubishi Kagaku Bio-Clinical Laboratories Inc., Tokyo, JAPAN; 2Department of Oral Surgery School of Medicine Tokai University, Kanagawa, JAPAN

Oral streptococci and *Aerococcus* have been implicated as causative pathogens of subacute endocarditis and bacteremia. In the present study, we found high level aminoglycoside-resistant (HLAR) strains among blood culture isolates of oral streptococci and *Aerococcus*. Seventy-nine isolates of oral streptococci and *Aerococcus* between December 1997 and January 2000 were tested to identify for HLAR bacteria by incubation in BHI agars containing 50μg/mL and 100μg/mL of gentamicin at 35°C for 24 hours. The MICs of gentamicin for the organisms grown on the agar were determined by the agar dilution method according to the M7-A5 guidelines established by the NCCLS. Two isolates with MICs of ~1024μg/mL were selected as possibly HLAR. Another two isolates with MICs of ~256μg/mL were also selected as HLAR-like isolates, although the resistance levels of these isolates were below the MICs listed for HLAR Enterococci in the NCCLS guideline. These four isolates were identified by using api strep 20 as two isolates each of *Streptococcus oralis* and *Aerococcus viridans*, and two isolates of *Streptococcus uberis*.

After the PCR amplification was performed, the DNA-sequence of the structural gene encoding 6'-acetyltransferase-2"-phosphotransferase (6'AAC-2" APH) in these four isolates was investigated according to the method for *Enterococcus faecalis* originally described by Ferretti *et al.* The results of the gene analysis showed that the DNA sequence of *A. viridans* No. 70, which had the highest MIC, ~1024μg/mL, matched that of *E. faecalis*, whereas no PCR-product was obtained from the other three isolates tested, although there is no definite proof of resistance mechanisms. In conclusion, it can be anticipated that this resistance trait has the potential of further spread among oral streptococci and *Aerococcus*.

D-41. Persistence of Small Colony Variant (SCV) and/or Normal *S. aureus* in Respiratory Secretions of Cystic Fibrosis (CF) Patients: Results of a 6 Year Prospective Study

B. C. Kahl1, A. Duebbers2, G. Lubritzki, B. Ritzerfeld1, M. Reilly1, E. Harms2, M. Herrmann1, C. Peters1
1Medical Microbiology, University of Muenster Hospital and Clinics, Muenster, GERMANY; 2Department of Pediatrics, University of Muenster Hospital and Clinics, Muenster, GERMANY

Background: S. aureus SCVs have been isolated from patients with recurrent osteomyelitis and endocarditis and have been associated with persistent infection. CF patients are often infected persistently with S. aureus. Recently, we demonstrated a high prevalence and persistence of SCV S. aureus in bronchial secretions of CF patients in a 34 month study. To specifically investigate the potential for long-term persistence of S. aureus SCVs in CF, we extended and reevaluated the prospective study after 72 months. Methods: Specimens were cultured on Columbia, Endo agar and additionally on BHI and Schaedler agar to grow SCV isolates. Species identification was done by standard methods and API ID 32 Staph. All SCV and normal isolates were analyzed by pulsed-field gel electrophoresis. The patients were characterized by age, Shwachman score and co-infection with Pseudomonas aeruginosa. Results: The data of 73 patients were evaluated. The patients were divided into 3 groups according to the culture of i) only normal S. aureus (28/73, 38.35 %), ii) SCV and/or normal S. aureus (21/73, 28.76 %) and iii) no S. aureus (24/73, 32.87 %). Almost all patients (19/21) with SCVs were treated with Trimethoprim/Sulfamethoxazole for more than 18 months. The majority of patients of group 1 and 2 were infected with one predominant clone with a low turnover of strains. SCV and/or normal S. aureus persisted up to 70 months with a mean persistence of 29.9 months for group 1 and 46.3 months for group 2, respectively. The comparison of the relative persistence (persistence/observation period) did not differ significantly between group 1 and 2. Patients with SCV S. aureus were significantly older, the clinical scores were lower indicating advanced disease and there was a trend of more frequent co-infection with P. aeruginosa compared to patients with only normal S. aureus. Conclusion: Isogenic SCV and normal S. aureus persisted for months or years in the lung of CF patients. SCV isolates were recovered from older patients with a more advanced clinical status of respiratory insufficiency after long-term TMP/SMX therapy.

D-42. Relationship Between the Polysaccharide Intercellular Adhesin (PIA) and Poly-N-Succinyl b-1-6 Glucosamine (PNSG) Molecules Produced by Pathogenic Staphylococci

T. Maira-Litran1, D. McKenney1, K. Pouliotti, A. Kropec1, Y. Wang1, D. A. Goldmann2, G. B. Pier1
1Brigham and Women's Hospital-Harvard Medical School, Boston, MA; 2Children's Hospital-Harvard Medical School, Boston, MA

Staphylococcus aureus and S. epidermidis are the most common bacterial isolates from nosocomial infections. Two related polysaccharides produced by proteins encoded by the intercellular adhesin locus (ica) have been chemically characterized-PIA, which is poly-N-acetyl-b-1-6 glucosamine and PNSG, which contains 10-30% acetate and 70-90% succinate substituents on the amino moiety of glucosamine. PNSG is an environmentally regulated, in vivo-expressed surface polysaccharide made by S. epidermidis and S. aureus. We compared the production of PIA and PNSG antigens by S. aureus MN8m, a strain hyperproducing PIA/PNSG isolated in our laboratory. Cultures were grown in chemically-defined media (CDM) for 24 hours maintaining neutral pH and aerating the culture with O2 at 2 L/min. Cell-free supernatants were used for PIA/PNSG isolation and purification, which was then analyzed by proton nuclear magnetic resonance (1H NMR). Antigens suspended in D2O for analysis appeared to be mainly PIA with no succinate signals detected. Similar results were obtained after samples were hydrolyzed with 1 or 2 M deuterated chloride (DCl). However, when extracts were hydrolyzed with 4 M DCl at 100 oC for 16 hours, succinate was detected in all samples. The succinate signal showed up as a singlet peak at approximately 2.7 ppm in the 1H NMR, indicative of free succinate. This is due to hydrolysis of PNSG and solubilization of succinate by strong acid. ELISA analysis on PIA/PNSG antigens using antisera raised to chemically derived PNSG lacking any acetate and chemically derived PIA lacking detectable succinate showed that both sera bound strongly to native and chemically derived antigens, as well as to the poly-glucosamine backbone, indicating substantial cross reactivity among these related antigens. Detection of the succinate residues in PNSG requires hydrolysis of antigen in strong acid prior to analysis by 1H NMR. PIA and PNSG polysaccharides are either mixtures of two separate molecules or are the same material wherein detection of the succinate component requires special analytic conditions.

D-43. The *Staphylococcus aureus* Global Regulators (agr and sar) Promote Severe Pneumonia in Mice

K. A. Overheim, J. W. Simecka, L. A. Tabor, M. E. Hart
University of North Texas Health Science Center, Fort Worth, TX

Staphylococcus aureus is a gram-positive bacterium and a leading cause of nosocomial infections. Among the nosocomial infections caused by *S. aureus*, pneumonia represents one of the more life-threatening diseases, particularly in the elderly population. Although *S. aureus* produces numerous toxins that contribute to various diseases, little is known about the involvement of these toxins in causing pneumonia. We hypothesize that toxins contribute to the severity of staphylococcal pneumonia, and that approaches that block toxin production or their effects will have a tremendous therapeutic effect. To begin to examine the role of toxin production on pneumonia, we took advantage of the fact that production of many toxins is regulated by at least two genetic loci, the accessory gene regulator (*agr*) and the staphylococcal accessory regulator (*sar*). Mutations in either or both of these regulators result in reduced levels of expression for many of the toxins with a concomitant reduction in virulence in several animal models of disease. To investigate the roles of these regulators in staphylococcal pneumonia, Balb/c mice were intranasally inoculated with *S. aureus* RN6390 or its isogenic mutants RN6911 (*agr*) and ALC488 (*sar*). Greater than 90% of the mice given 10⁸ colony forming units of the parent strain of *S. aureus* died within 2 days after infection. In contrast, about 50% of mice similarly infected with *sar* mutant survived, whereas all mice infected with *agr* mutant survived. The difference in virulence was not due to the number of bacteria surviving in the lung. Also, bacterial dissemination to extrapulmonary sites was not the cause for lethality of the organism. These studies demonstrate that the severity of staphylococcal pneumonia in a mouse model is increased with a functional *agr* and *sar* regulatory system, and supports a role for toxin production in the pathogenesis of a life-threatening lung disease.

D-44. The Role of the Capsular Polysaccharide Adhesin (PS/A) Virulence and Immunoprophylaxis of *S. aureus* Endocarditis in Rats

D. McKenney1, K. L. Pouliotti, T. Maira-Latran1, A. Kropec1, S. E. Cramton2, F. Goetz2, D. A. Goldmann3, G. B. Pier1
1Channing Laboratory, Boston, MA; 2Mikrobielle Genetik, Tuebingen, GERMANY; 3Childrens Hospital, Boston, MA

We have previously reported that the *intercellular adhesion locus (ica)* found in both *S. epidermidis* and *S. aureus* encodes proteins that synthesize PS/A and polysaccharide intercellular adhesin (PIA). These polysaccharides have the potential to be broadly protective vaccines against staphylococcal infections. Prior results showing protection against *S. aureus* in a mouse renal infection model were extended to investigate the role of PS/A in virulence in a rat model of endocarditis and the protective efficacy of PS/A in this model following active and passive immonization. Endocardial infection was induced by IV or IP injection of rats that had been catheterized via the carotid artery with a silastic catheter placed through the aortic valve. Rats were then infected IV with *S. aureus* 10833, *S. aureus* 10833Δ*ica*, a PS/A negative mutant and *S. aureus* 10833Δ*ica* (pSC38) which contains a plasmid containing the intact *ica* locus. After 7 days, rats that survived the infection period were assessed for infected valve vegetations. Infectious dose-50 (ID_{50}) values were calculated for each strain. Rats infected with wild-type 10833 cells had an ID_{50} of less than 43 CFU compared to 6.9 x 10⁶ CFU using the 10833Δ*ica* mutant. The *ica*-complemented strain had an ID_{50} of less than 120 CFU. 10 of 14 rats died when infected with wild-type 10833 compared to 0/16 rats infected with the 10833Δ*ica* strain (P=.00003). Protection studies showed that 1/9 rats actively immunized with PS/A were infected after challenge with the 10833 wild-type strain compared to 7/8 immunized with an irrelevant polysaccharide (P=.002). Rats passively immunized with antibodies to PS/A and challenged with wild-type strain 10833 were protected against infection (3/7 positive) compared to animals given an irrelevant hyperimmune serum (7/7 positive, P=.03). Antibodies to PS/A did not protect against infection with strain 10833Δ*ica* when a challenge dose sufficient to cause endocarditis was used. These results point to an important role of PS/A in virulence of *S. aureus* endocarditis and of the potential efficacy of antibodies to this material in preventing endocardial infection.

D-45. Insights Into the Immunology of Acute Rheumatic Fever: A Study of Cytotoxicity and the TH1/TH2 Paradigm

M. W. Cunningham1, A. Beeker1, J. S. Heuser1, H. Hill2, N. Augustine2, G. Veasy2, E. E. Adderson3
1University of Oklahoma Health Sciences Center, Oklahoma City, OK; 2University of Utah School of Medicine, Salt Lake City, UT; 3St. Jude Children's Hospital, Memphis, TN

Acute rheumatic fever (ARF) is an autoimmune sequela of group A streptococcal infection which has multiple manifestations including carditis, chorea, and arthritis. Antibodies and T cells responsive to streptococcal and host antigens produce inflammatory responses in tissues and lead to disease manifestations. To investigate mechanisms in the pathogenesis of ARF, more than 22 sera were studied to test the hypothesis that complement-mediated cytotoxicity as well as differences in IgG subclass responses to M protein and the group A carbohydrate epitope N-acetyl-glucosamine (GlcNAc) were associated with different manifestations of disease. Cytotoxicity in chromium-51 release assays was observed in approximately 45 percent of these sera and correlated with the presence of carditis. Comparison of IgG subclass responses in different manifestations of ARF revealed striking differences. IgG1 and/or IgG3 subclass responses against M protein (PepM5) as measured in the ELISA were elevated (300-500ug/ml) in carditis and/or chorea and not in pure arthritis (50ug/ml). In pure chorea, IgG1 and IgG3 subclasses were lower (100ug/ml) unless associated with carditis. IgG2 responses in carditis and chorea were predominantly against GlcNAc (150ug/ml). In contrast, in arthritis IgG1 responses were found against GlcNAc. To summarize, carditis sera were cytotoxic with elevated IgG1 and IgG3 against M protein and IgG2 against GlcNAc. Chorea was similar to carditis in IgG2 responses to GlcNAc but had lower IgG1 and IgG3 against M protein. Pure arthritis was very different from carditis and chorea in that arthritis had IgG1 directed against GlcNAc. In humans, IgG1 and IgG3 are associated with opsonizing and cytotoxic antibody and with TH1 responses and delayed type hypersensitivity. Cytotoxicity and IgG1 and IgG3 reponses against M protein (PepM5) appear to correlate with carditis and permanent damage to the heart valve. The evidence supports a TH1 mechanism of pathogenesis in carditis. Although chorea and arthritis may be driven by a TH2 mechanism, more study will be required to prove such a concept.

D-46. A Conditional Lethal Mutation in the YycF Essential Response Regulator of *Staphylococcus aureus* Reveals a Novel Calcium-Dependent Growth Requirement

P. K. Martin, A. Arquiza, J. M. Buysse
Microcide Pharmaceuticals, Inc, Mountain View, CA